



5-Ethynylarylnaphthalimides as antitumor agents: Synthesis and biological evaluation



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ABSTRACT

A set of 5-ethynylarylnaphthalimides was synthesized by Sonogashira cross-coupling reactions and evaluated for antiproliferative and antitopoisomerase II *in vitro* activities. Furthermore docking studies of these molecules as DNA-intercalators were carried out and the *in vivo* DNA-damaging activity was also determined with the model organism *Saccharomyces cerevisiae*. From the obtained results three naphthalimides **6**, **13** and **14** showed strong topoisomerase II inhibitory activity. These three molecules also presented good docking scores as DNA-intercalators using a self-complementary oligodeoxynucleotide d(ATGCAT)₂ as a model, and compounds **13** and **14** were among the most cytotoxic in the *in vivo* DNA-damaging activity.

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1. Introduction

Small-molecule-mediated DNA targeting represents one of the most effective approaches for the development of chemotherapeutics. Naphthalimide derivatives constitute interesting scaffolds because of their well characterized DNA-binding properties.^{1,2} Amonafide (Fig. 1) is one of the most widely studied naphthalimides, and its antitumor activity seems to be related to poisoning the nuclear enzyme topoisomerase II to generate DNA-strand breaks.^{3,4} Amonafide showed excellent activity in clinical phase II breast cancer trials, but it failed in clinical phase III trials because of its unpredictable side effects on dose-limiting bone marrow toxicity due to its metabolization to N-acetyl-amonafide.^{5,6} To avoid the side effect of amonafide several strategies have been carried out. Thus, several N-substituted amonafide derivatives were synthesized by Kiss⁷ and Quian.⁸ Some heterocyclic-fused naphthalimides such as thiazonaphthalimides,^{9–11} or benzopyran-naphthalimides¹² were also prepared. Other approach was the preparation of 5-non-amino aromatic substituted naphthalimides.^{13,14} In this sense our group prepared and designed a series

of arylnaphthalimides,¹⁴ and we found that all synthesized naphthalimides but those carrying H-bond donor groups, strongly inhibited Topo II, and they were likely generating DNA double strand breaks (DSB) *in vivo* as determined by assays with the model organism *Saccharomyces cerevisiae*. These compounds were able to arrest yeast cells in G2, promote the formation of Rad52 foci *in vivo* and made *rad52* mutants hypersensitive. Based on these previous results and because amonafide, together with cytarabine has been introduced into phase III clinical trial against secondary acute myeloid leukemia¹⁵ we encouraged the study of antitumor properties of new 5-ethynylarylnaphthalimides, since the acetylene bond itself is believed to improve the intercalating properties.¹⁶ Thus the naphthalimide moiety can be attached to a variety of substituted aromatic rings via an acetylene bridge, which provides the necessary structural rigidity and twisting ability and still unites the aromatic structures.

2. Results and discussion

Naphthalimides (**4–14**) were synthesized from the commercial 1,8-naphthalic anhydride (**1**) which was quantitatively and selectively converted into 3-iodo-1,8-naphthalic anhydride (**2**) by using of iodine in the presence of Ag₂SO₄/H₂SO₄ (Scheme 1).¹⁴ The treatment of **2** with N,N-dimethyl-ethane-1,2-diamine yielded

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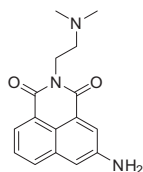
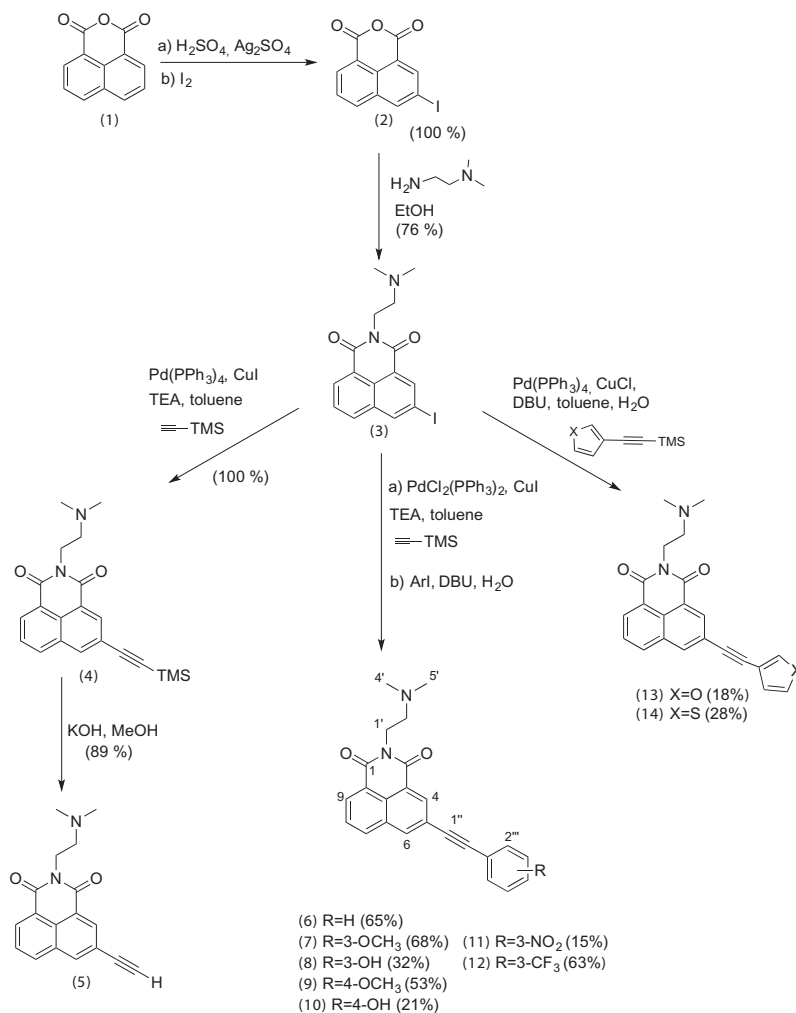


Fig. 1. Structure of amonafide.

the corresponding imide (**3**) in 76% yield. The derivative **4** was obtained by Sonogashira cross-coupling reaction using **3** in the presence of trimethylsilylacetylene (TMSA) (1 equiv) in anhydrous toluene, TEA (6 equiv), Pd(PPh₃)₄ and CuI (10 mol% each). Deprotection of TMS group to yield the terminal alkyne **5** was achieved using 1 M solution of KOH in MeOH. The

arylethynynaphthalimides (**6–12**) were obtained from **3** through *in situ* Sonogashira cross-coupling reactions.¹⁷ Thus, **3** dissolved in dry toluene was treated with trimethylsilylacetylene (TMSA) (1 equiv), TEA (6 equiv), CuI (10 mol%), PdCl₂(PPh₃)₂ (6 mol%) in the dark. After disappearance of **3**, sequential addition of 1.0 equiv of the corresponding aryl iodide, DBU (12 equiv) and 40 mol% of water gave rise to the corresponding naphthalimides. The ethynynaphthalimides with furan (**13**) and thiophene (**14**) rings were synthesized in an alternative way from **3** by using the corresponding (furan-3-ylethynyl)trimethylsilane and (thiophene-3-ylethynyl)trimethylsilane in the presence of CuCl (50 mol%), DBU (12 equiv) and 10 mol % of Pd(PPh₃)₄.

Some physicochemical descriptors for the synthesized naphthalimides (**6–14**) were calculated and the results are shown in Table 1. As we can see all of them show acceptable requirements for druggability.



Scheme 1. Synthesis of ethynynaphthalimides (**6–14**).

Table 1
 Calculated physicochemical descriptors of compounds **6–14**.^a

Property	Optimal range	6	7	8	9	10	11	12	13	14
MW	<500	368	440	440	384	349	413	372	358	374
Log P	<5	3.47	4.69	4.71	2.96	2.99	3.40	4.34	2.41	3.06
H-bond donors	<5	0	0	0	1	1	0	0	0	0
H-bond acceptors	<10	4	5	5	5	5	7	4	5	4
Rotable bonds	<5	3	5	5	3	3	4	4	3	3
TPSA	<140	42.31	51.55	51.55	62.54	62.54	88.14	42.31	55.45	42.31

^a Values were calculated using Molinspiration Cheminformatics Software (2015), (<http://www.molinspiration.com>).

Table 2
Antiproliferative activity of aryl-naphthalimides (**5–14**) against the human SK-Br-3, HL60 and HEL cell lines.^a

Compound	SKBr-3	HL-60	HEL
5	>10	10.0 ± 0.9	8.1 ± 0.08
6	4.6 ± 0.1	4.6 ± 0.2	1.4 ± 0.06
7	2.0 ± 0.1	1.0 ± 0.04	1.0 ± 0.05
8	>30	10.2 ± 0.6	1.2 ± 0.06
9	2.1 ± 0.1	1.1 ± 0.06	2.0 ± 0.1
10	2.1 ± 0.06	5.7 ± 0.1	13.1 ± 0.1
11	>10	13.6 ± 0.6	21.7 ± 0.1
12	0.1 ± 0.02	2.3 ± 0.08	2.2 ± 0.06
13	9.6 ± 0.4	3.5 ± 0.1	5.6 ± 0.3
14	>10	5.4 ± 0.3	5.0 ± 0.08
Amonafide	7.4 ± 0.9	3.4 ± 0.5	4.0 ± 1.2

^a Expressed as IC₅₀ values given in μM and determined as means ± SD (n = 3).

The obtained arylolethynyl-naphthalimides were evaluated *in vitro* against SK-Br-3 (Human Breast Cancer), HL60 (Human Promyelocytic leukemia) and HEL (Human Erythroleukemia) tumor cell lines. The results obtained are shown in Table 2. The importance of the ethynylphenyl moiety for the antiproliferative activity resulted evident since compound **5** with a terminal alkyne showed a loss of activity (compare **5** vs **6**). Introduction of electron-donating groups such as methoxy and hydroxyl groups at the *para*- or *meta*-position of the phenyl ring retained activity (compounds **7**, **9**, and **10**) but not in all cases (i.e. **8** for the SKBr-3 and HL-60 cell lines), as compared to compound **6**. The highest antiproliferative activity was obtained with the introduction of the electron-withdrawing group CF₃ (compound **12**). The nitro derivative **11** showed lower antiproliferative activity than **6** and amonafide. Substitution of the phenyl group with

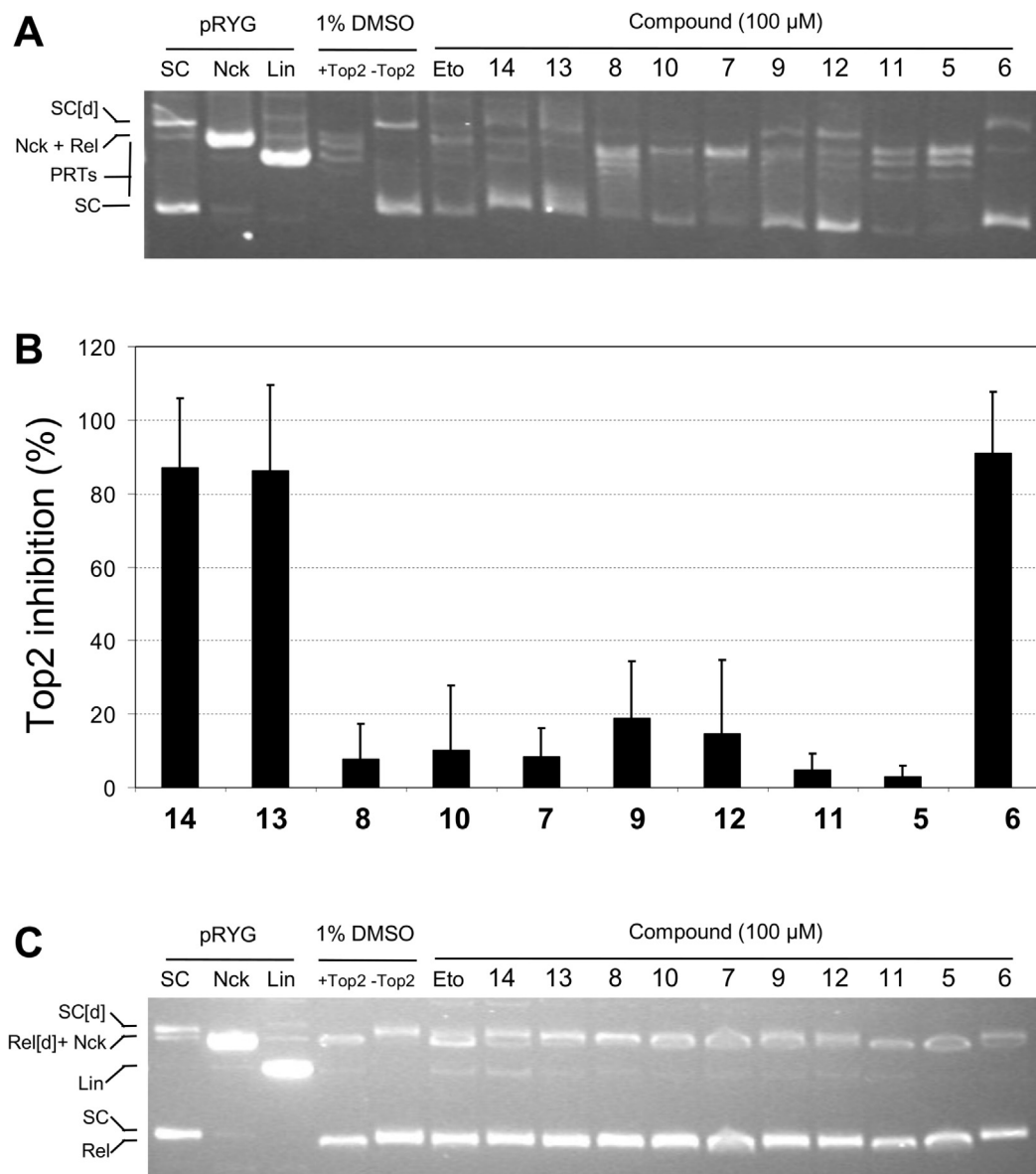


Fig. 2. Topo 2-mediated relaxation and cleavage assays of supercoiled circular DNA upon incubation with naphthalimides (**6–14**). (A) Agarose electrophoresis run in the absence of ethidium bromide to separate substrates (SC form) and products (relaxed topoisomers) of the hTopo II α relaxation reaction. Covalently closed negatively supercoiled pRYG plasmid (SC form) was treated with 2U hTopo II α in the presence of 100 μM of each naphthalimide in 1% (v/v) DMSO. (B) Quantitation of percentage of hTopo II α inhibition of four independent relaxation assays (mean ± SD) for each naphthalimide. (C) Agarose electrophoresis run under the presence of ethidium bromide to separate substrates (SC form) and intermediate products (linearized plasmid) of the hTopo II α cleavage assay. Covalently closed negative supercoiled pRYG plasmid (SC form) was treated with 10U hTopo II α in the presence of 100 μM of each naphthalimide in 1% (v/v) DMSO. Etoposide (Eto) was included as a positive control. Nck (nicked plasmid), Rel (fully relaxed plasmid), PTRs (partially relaxed plasmid topoisomers), SC (supercoiled plasmid), SC[d] (SC form of the plasmid dimer), Rel[d] (fully relaxed dimer).

heterocyclic rings such as furan or thiophene (compounds **13** and **14**) led to similar amonafide's values. Thus, some of the 5-ethynylaryl derivatives exhibited good and higher antiproliferative activities than amonafide. In the case of compound **12** the introduction of the acetylene group produced an increased antiproliferative activity respect to the corresponding 5-(3-trifluoromethylphenyl)naphthalimide analogue (SKBr-3 IC_{50} $1.2 \pm 0.3 \mu M$ /HEL IC_{50} $1.6 \pm 0.2 \mu M$) previously reported by us.¹⁴

Next, we carried out anti-Topoisomerase II assays for this set of compounds and the results are shown in Fig. 2. Compounds **6**, **13**, and **14** strongly inhibited Topo II, consequently for this series of naphthalimides the presence of a heteroaromatic ring (**13**, **14**) and, an unsubstituted aryl ring produced the best results. Probably these compounds could act as DNA intercalators since the supercoiled form of the DNA substrate appeared shifted after being incubated with these compounds. Compound **12** having the best antiproliferative activity showed a low inhibition of Topo II. Finally, and as we can see in the Fig. 2C, **14** happened to be a Topo II poison comparable to etoposide.

In order to predict the binding site along with preferred orientation of the molecules inside the DNA and to explore the structural determinants responsible for the better intercalating properties of some aryethynyl naphthalimides, molecular modeling studies were performed. The DNA-ligand binding affinity and DNA intercalating poses of these aryethynyl naphthalimides were predicted using molecular docking methodology in Glide software with default settings.^{18,19} For this study the crystal structure of the self-complementary oligodeoxy nucleotide d(ATGCAT)₂ was used as a model.^{20–22} The analysis of docking study revealed that all the synthesized ligands interact with DNA via an intercalation mode involving π - π stacking interactions and hydrogen-bonds between the functional groups present in the naphthalimide and the DNA base pairs. The highest docking scores were observed in the range from -8.76 to -7.13 kcal mol⁻¹. The common structural portion constituted by a polycyclic system with a substituent aryethynyl group is fundamental not only for the lineup of the complex with the biological target if not also for keeping a similar binding pattern as previously reported.¹⁴ The ligands fit well into the intercalative mode of the targeted DNA and most of them share a similar

binding mode, except those compounds with a furan ring (**13**) or thiophene ring (**14**) where the preferred orientation of the ligand changes into the DNA. A similar orientation is adopted when the aromatic ring is substituted in meta-position by a trifluoromethyl group (**12**). In this sense, for example, the planar portions of the structure of the derivative **14** fit into the DNA in a parallel fashion through a base pairs intercalation establishing π - π stacking interactions with DNA aromatic rings (Fig. 3). The aminoalkyl chain establishes Van der Waals contacts and the strong hydrogen bonding interactions were observed between the amino groups, which are protonated at neutral pH with N7 of G3.

In order to explore whether the ethynylarylnaphthalimides were causing DNA damage *in vivo*, we carried out dose-response growth assays with the model organism *Sacchararomyces cerevisiae* (Fig. 4). We used a reference strain proficient for the DNA damage response, and a derivative that bears a gene knockout (*rad52Δ*) that hypersensitizes against the kind of DNA damages expected from anti-TopII poisons (i.e., DNA double strand breaks and replication stress). All compounds were capable of inhibiting growth within the low micromolar range (between 5 and 100 μM), except for **10** in the reference strain. In most cases, the *rad52Δ* strain was more sensitive than the reference strain, confirming that DNA damage was a major cause of cytotoxicity. Besides, ethynylarylnaphthalimides **13** and **14** were among the most cytotoxic, thus correlating well with the anti-Topo II *in vitro* results.

Overall, there was a good correlation between DNA binding predictions, *in vitro* topoisomerase II inhibition, and *in vivo* cytotoxicity in *S. cerevisiae* (e.g., **6**, **13** and **14**). Remarkably though, antiproliferative activity against the tumor cell lines showed that other compounds such **7**, **9** and **12** performed better despite being modest anti-Top2 agents. It is interesting to speculate that naphthalimides derivatives that are weaker DNA intercalators might be more available for new targets where to exert novel and potent antitumor activities. In this respect, it has shown that non-intercalating naphthalimides promote senescence;⁷ whereas substituents like polyamines can target naphthalimides towards new organelles or molecular targets (e.g., Akt/mTOR).²³

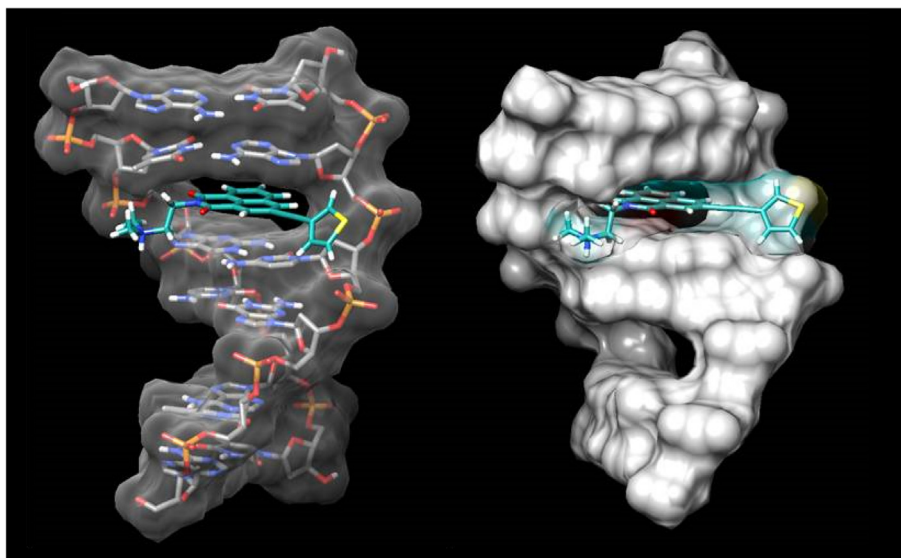


Fig. 3. Preferred orientation of compound **14** into DNA, in a parallel fashion to the DNA bases and establishing π - π stacking interactions with nucleotides. The figure also shows the interaction of the amino groups of **14** with N7 of G3 through hydrogen bonds.

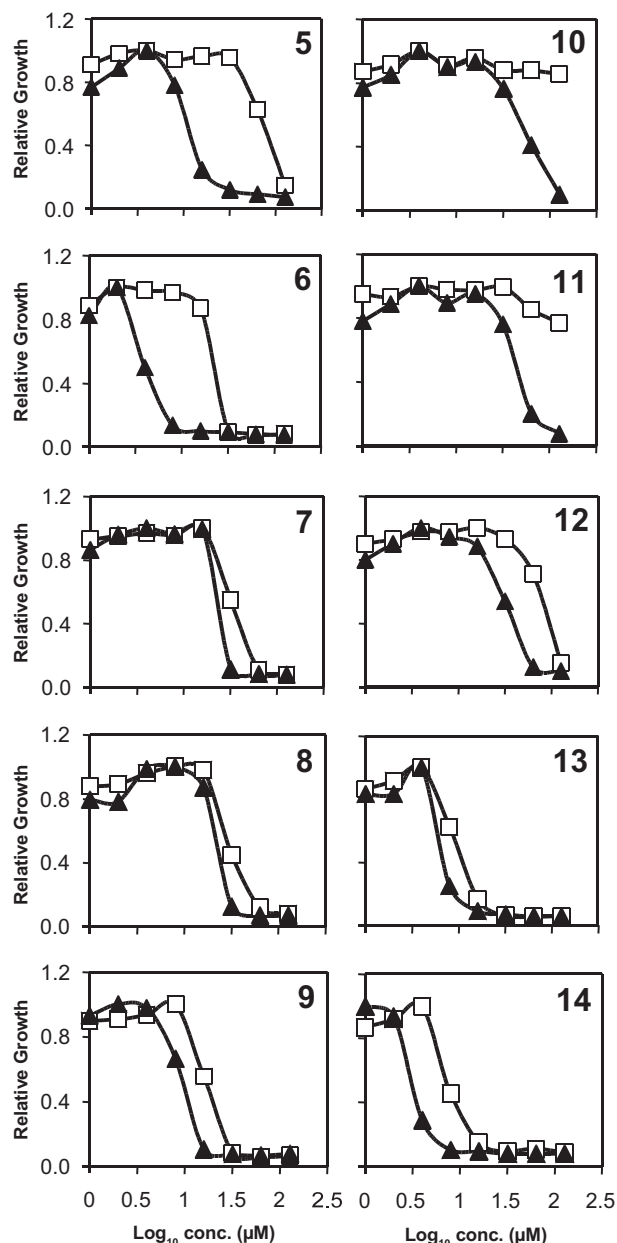


Fig. 4. Sensitivity to the novel set of ethynylarylnaphthalimides of wild type and homologous recombination deficient yeast cells. Strains proficient (*RAD52*) and deficient (*rad52Δ*) for the homologous recombination pathway were grown for 24 h under increasing concentrations of the tested ethynylarylnaphthalimides. Relative growth to a control with DMSO 1% (v/v) was plotted against the drug concentrations. In the graph, open squares depict growth of the *RAD52* strain and filled triangles depict the *rad52Δ* isogenic counterpart.

3. Conclusions

Based on previous results on 5-arylnaphthalimides a set of novel 5-ethynylarylnaphthalimides (**6–14**) has been synthesized from 2-(2-(dimethylamino)ethyl)-5-iodo-1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-dione (**3**) through *in situ* Sonogashira cross-coupling reactions. The obtained aryethynyl naphthalimides were evaluated *in vitro* against SK-Br-3, HL60 and HEL tumor cell lines. The highest antiproliferative activity was obtained with the introduction of the electron-withdrawing group CF₃ (**12**), and some of the 5-ethynylaryl derivatives exhibited higher antiproliferative activities than amonafide. Anti-Topo II assays for this set of compounds determined that compounds **6**, **13**, and **14** strongly inhibited human

Topo II α . Since the supercoiled form of the DNA substrate appeared shifted after being incubated with these compounds, they could act as DNA intercalators, which was supported by Docking studies. The *in vivo* DNA-damaging activity was also determined with the model organism *Saccharomyces cerevisiae*, and ethynylarylnaphthalimides **13** and **14** were among the most cytotoxic, thus correlating well with the anti-Topo II *in vitro* results. Since the physicochemical descriptors of these compounds do not violate the optimal requirements for druggability, these naphthalimides are promising molecules for further research.

4. Experimental section

4.1. General methods

NMR spectra were recorded in CDCl₃ or C₆D₆ at 400 or 500 MHz for ¹H NMR and 100 or 150 MHz for ¹³C NMR. Chemical shifts are given in (δ) parts per million and coupling constants (*J*) in hertz (Hz). ¹H and ¹³C spectra were referenced using the solvent signal as internal standard. HREIMS were recorded using a high resolution magnetic trisector (EBE) mass analyzer. Analytical thin-layer chromatography plates used were POLYGRAM-SIL G/UV254. Preparative thin-layer chromatography was carried out with Analtech GF plates (20 × 20 cm, 1000 μ m) using appropriate mixtures of ethyl acetate and hexanes. All solvents and reagents were purified by Standard techniques reported²⁴ or used as supplied from commercial sources. Compounds **2** and **3** were synthesized as described in Ref.¹⁴ All compounds were named using ACD40 Name-Pro program, which is based on IUPAC rules.

4.1.1. Preparation of 2-(2-(dimethylamino)ethyl)-5-((trimethylsilyl)ethynyl)-1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-dione (**4**)

49 mg of **3** (0.12 mmol) were dissolved in 5 mL of anhydrous toluene, and then in order Pd(PPh₃)₄ (10 mol%, 13.9 mg, 0.012 mmol), and CuI (10 mol%, 2.3 mg, 0.012 mmol) were added under an argon atmosphere. Then, 0.7 μ L of TEA (4 equiv, 0.48 mmol) were added followed immediately by 0.67 μ L of TMSA (4 equiv, 0.48 mmol). The reaction mixture was stirred at room temperature until disappearance of the starting naphthalimide **3** (0.5 h). Next, the reaction mixture was filtered and the solvent was removed under reduced pressure affording the crude product as a brown solid. The crude was dissolved in hot EtOH and compound **4** precipitated as a yellow solid. ¹H NMR (CDCl₃) δ 0.02 (9H, s, CH₃-TMS), 2.35 (6H, s, H-5', H-4'), 2.68 (2H, t, *J* = 5.6 Hz, H-2'), 4.32 (2H, t, *J* = 5.6 Hz, H-1'), 7.76 (1H, t, *J* = 6.2 Hz, H-8), 8.15 (1H, d, *J* = 6.5 Hz, H-7), 8.32 (1H, d, *J* = 0.9 Hz, H-6), 8.60 (1H, d, *J* = 6.0 Hz, H-5), 8.62 (1H, d, *J* = 1.0 Hz, H-4); ¹³C NMR (CDCl₃) δ 38.0 (CH₂, C-1'), 45.6 (CH₃, C-5', C-4'), 56.8 (CH₂, C-2'), 97.2 (CH, C-2''), 103.1 (C, C-1''), 122.1 (C, C-3a), 122.4 (C, C-9a), 122.5 (C, C-5), 127.1 (C, C-9b), 127.4 (CH, C-8), 131.0 (C, C-6a), 131.4 (CH, C-9), 133.3 (CH, C-7), 133.7 (CH, C-4), 136.3 (CH, C-6), 163.1 (C, C-1), 163.6 (C, C-3); EIMS *m/z* (%) 364 (16), 349 (20), 320 (10), 191 (2), 71 (41), 58 (100); HREIMS 364.1614 (calcd for C₂₁H₂₄N₂O₂Si [M]⁺364.1607); IR ν_{\max} 2953, 2784, 2303, 1696, 1658, 1595, 1460, 1426, 1328, 1240, 1169, 1143, 1024, 981, 931, 910, 841 cm⁻¹.

4.1.2. Preparation of 2-(2-(dimethylamino)ethyl)-5-ethynyl-1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-dione (**5**)

To 0.25 g (0.68 mmol) of compound **4** dissolved in 30 mL of MeOH were added 16 mL of a 1 M KOH solution, and the reaction mixture was stirred at room temperature for 15 min. Then it was treated with a 3 N HCl solution until neutral Ph. The solution was extracted with diethyl ether (3 × 20 mL) and the organic phases were collected and dried over Na₂SO₄. The solvent was removed under vacuum to afford **5** in 89% yield. ¹H NMR (CDCl₃) δ 2.35

(6H, s, H-5', H-4'), 2.64 (2H, t, $J = 6.9$ Hz, H-2'), 3.29 (1H, s, H-2''), 4.30 (2H, t, $J = 6.9$ Hz, H-1'), 7.73 (1H, t, $J = 7.7$ Hz, H-8), 8.10 (1H, d, $J = 8.1$ Hz, H-7), 8.25 (1H, s, H-6), 8.53 (2H, bs, H-9, H-4); ^{13}C NMR (CDCl_3) δ 38.2 (CH_2 , C-1'), 45.7 (CH_3 , C-5', C-4'), 56.9 (CH_2 , C-2'), 79.6 (CH , C-2''), 81.9 (C, C-1''), 121.1 (C, C-9a), 122.6 (C, C-3a), 122.8 (C, C-5), 126.8 (C, C-9b), 127.5 (CH, C-8), 131.1 (C, C-6a), 131.7 (CH, C-4), 133.4 (CH, C-7), 133.8 (CH, C-9), 136.9 (CH, C-6), 163.3 (C, C-1 or C-3), 163.7 (C, C-3 or C-1); HREIMS 292.1245 (calcd for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_2$ $[\text{M}]^+$ 292.1212); IR ν_{max} 3248, 2953, 2815, 2773, 1699, 1658, 1626, 1592, 1424, 1326, 1257, 1236, 1137, 1021, 928, 907, 852, 781 cm^{-1} .

4.1.3. General procedure for the preparation of arylethynylphthalimides (**6–12**)

A 10 mL round bottom flask fitted with a rubber septum was purged with dry argon, and charged with $\text{PdCl}_2(\text{PPh}_3)_2$ (1.6 mg, 6 mol%), CuI (1.52 mg, 10 mol%) and iodonaphthalimide **3** (31.5 mg, 0.08 mmol) dissolved in dry toluene (0.2 M, 0.4 mL). Argon-sparged NEt_3 (66.9 μL , 6 equiv) and trimethylsilylethynylene (11.9 μL , 1.05 equiv) were then added by syringe. The reaction flask was covered in aluminum foil and left stirring at a high rate of speed (0.5 h). Then the corresponding iodoaryl derivative (1 equiv), DBU (0.15 mL, 12 equiv) and distilled water (40 mol%) were added. The mixture was stirred at room temperature until disappearance of the aryl derivative, and then it was partitioned in ethyl ether and distilled water. The organic layer was washed with 10% HCl, saturated aqueous NaCl, dried over MgSO_4 , filtered and the solvent removed under vacuum. The crude product is purified by alumina preparative-TLC.

4.1.4. 2-(2-(Dimethylamino)ethyl)-5-((phenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**6**)

Compound **6** was obtained from 62 mg of 3-iodonaphthalimide (0.16 mmol) following the general procedure described above after stirring for 8 h at room temperature. The crude product was purified by alumina preparative-TLC using EtOAc/hexanes (2:3) to yield 38 mg (65%) of compound **6** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.36 (6H, s, H-5', H-4'), 2.67 (2H, t, $J = 5.5$ Hz, H-2'), 4.34 (2H, t, $J = 5.5$ Hz, H-1'), 7.40 (3H, m, H-4'', H-5'', H-3''), 7.59 (1H, m, H-2'', H-6''), 7.76 (1H, t, $J = 6.2$ Hz, H-8), 8.16 (1H, d, $J = 6.5$ Hz, H-7), 8.33 (1H, s, H-6), 8.57 (1H, d, $J = 5.8$ Hz, H-9), 8.66 (1H, s, H-4); ^{13}C NMR (CDCl_3) δ 38.0 (CH_2 , C-1'), 45.6 (CH_3 , C-5', C-4'), 56.9 (CH_2 , C-2'), 87.8 (C, C-2''), 91.8 (C, C-1''), 122.4 (C, C-1''), 122.7 (C, C-3a), 122.9 (C, C-9a), 127.3 (C, C-9b), 127.5 (CH, C-4''), 128.5 (CH, C-5''), 128.9 (CH, C-8), 131.5 (CH + C, C-9 + C-6a), 131.8 (CH, C-2''), 133.5 (CH, C-7), 133.7 (CH, C-6), 136.0 (CH, C-4), 163.9 (C, C-3 or C-1), 163.6 (C, C-1 or C-3); EIMS m/z (%) 368 (28), 324 (10), 310 (2), 297 (7), 226 (6), 71 (21), 58 (100); HREIMS 368.1514 (calcd for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_2$ $[\text{M}]^+$ 368.1525); IR ν_{max} 757, 786, 1238, 1426, 1664, 1701, 2214, 2778, 2961, 3060, 3398 cm^{-1} .

4.1.5. 2-(2-(Dimethylamino)ethyl)-5-((3-methoxyphenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**7**)

Compound **7** was obtained from 31.5 mg of 3-iodonaphthalimide (0.08 mmol) following the general procedure described above after stirring for 8 h at room temperature. The crude product was purified by alumina preparative-TLC using EtOAc/hexanes (2:3) to yield 21.7 mg (68%) of compound **7** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.40 (6H, s, H-5', H-4'), 2.72 (2H, t, $J = 6.8$ Hz, H-2'), 3.84 (3H, s, OCH_3), 4.34 (2H, t, $J = 6.8$ Hz, H-1'), 6.93 (1H, dd, $J = 1.8, 8.2$ Hz, H-6''), 7.08 (1H, bs, H-2''), 7.16 (1H, d, $J = 7.7$ Hz, H-4''), 7.30 (1H, t, $J = 8.0$ Hz, H-5''), 7.73 (1H, t, $J = 7.7$ Hz, H-8), 8.12 (1H, d, $J = 8.0$ Hz, H-7), 8.34 (1H, d, $J = 1.1$ Hz, H-6), 8.58 (1H, d, $J = 7.2$ Hz, H-9), 8.67 (1H, d, $J = 1.3$ Hz, H-4); ^{13}C NMR (CDCl_3) δ 37.9 (CH_2 , C-1'), 45.5 (CH_3 ,

C-5', C-4'), 55.3 (CH_3 , OCH_3), 56.6 (CH_2 , C-2'), 87.6 (C, C-2''), 91.7 (C, C-1''), 115.5 (CH, C-4''), 116.5 (CH, C-2''), 122.3 (C, C-5), 122.6 (C, C-3a), 122.8 (C, C-9a), 124.3 (CH, C-6''), 127.5 (CH, C-5''), 128.4 (C, C-9b), 128.5 (C, C-1''), 129.5 (CH, C-8), 131.4 (C, C-6a), 131.5 (CH, C-4), 133.5 (CH, C-9), 133.7 (CH, C-6), 136.0 (CH, C-7), 159.4 (C, C-3''), 163.5 (C, C-1 or C-3), 163.9 (C, C-1 or C-3); EIMS m/z (%) 398 (23), 327 (4), 213 (4), 71 (25), 58 (100); HREIMS 398.1628 (calcd for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_3$ $[\text{M}]^+$ 398.1630); IR ν_{max} 545, 789, 900.23, 1046.74, 1249.29, 1340.91, 1429.60, 1666.93, 1705.56, 1964.62, 2838.01, 3068.18, 3416.22 cm^{-1} .

4.1.6. 2-(2-(Dimethylamino)ethyl)-5-((3-hydroxyphenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**8**)

Compound **8** was obtained from 62.0 mg of 3-iodonaphthalimide (0.16 mmol) following the general procedure described above after stirring for 8 h at room temperature. The crude product was purified by alumina preparative-TLC using EtOAc/hexanes (2:3) to yield 19.7 mg (32%) of compound **8** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.46 (6H, s, H-5', H-4'), 2.94 (2H, t, $J = 5.9$ Hz, H-2'), 4.44 (2H, t, $J = 5.5$ Hz, H-1'), 6.26 (1H, dd, $J = 1.4, 2.2$ Hz, H-2''), 6.38 (1H, ddd, $J = 1.0, 2.7, 8.3$ Hz, H-4''), 6.68 (1H, dt, $J = 1.2, 7.4$ Hz, H-6''), 6.82 (1H, t, $J = 7.9$ Hz, H-5''), 7.69 (1H, t, $J = 8.0$ Hz, H-8), 8.1 (1H, d, $J = 8.4$ Hz, H-7), 8.28 (1H, d, $J = 1.4$ Hz, H-6), 8.50 (1H, dd, $J = 1.0, 7.4$ Hz, H-9), 8.63 (1H, d, $J = 1.6$ Hz, H-4); ^{13}C NMR (CDCl_3) δ 37.7 (CH_2 , C-1'), 45.4 (CH_3 , C-5', C-4'), 57.2 (CH_2 , C-2'), 87.7 (C, C-2''), 91.8 (C, C-1''), 116.3 (CH, C-4''), 118.0 (CH, C-2''), 122.6 (C, C-5), 122.7 (C, C-3a), 122.8 (CH, C-6''), 122.9 (C, C-9a), 123.0 (C, C-1''), 127.2 (C, C-9b), 127.3 (CH, C-5''), 129.0 (CH, C-8), 131.4 (CH, C-4), 131.5 (C, C-6a), 133.4 (CH, C-9), 134.0 (CH, C-6), 136.0 (CH, C-7), 156.4 (C, C-3''), 164.1 (C, C-1 or C-3), 164.4 (C, C-1 or C-3); EIMS m/z (%) 385 (26), 377 (16), 277 (17), 150 (4), 71 (23), 58 (100); HREIMS 384.1467 (calcd for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_3$ $[\text{M}]^+$ 384.1474); IR ν_{max} 3595, 2958, 2920, 2852, 2203, 1695, 1654, 1601, 1574, 1512, 1423, 1275, 1159, 830, 744 cm^{-1} .

4.1.7. 2-(2-(Dimethylamino)ethyl)-5-((4-methoxyphenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**9**)

Compound **9** was obtained from 31.5 mg of 3-iodonaphthalimide (0.08 mmol) following the general procedure described above after stirring for 8 h at room temperature. The crude product was purified by alumina preparative-TLC using EtOAc/hexanes (2:3) to yield 16.8 mg (53%) of compound **9** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.36 (6H, s, H-5', H-4'), 2.67 (2H, t, $J = 6.8$ Hz, H-2'), 3.86 (3H, s, OCH_3), 4.33 (2H, t, $J = 6.8$ Hz, H-1'), 6.92 (2H, d, $J = 8.2$ Hz, H-2''), 7.53 (2H, d, $J = 8.2$ Hz, H-3''), 7.75 (1H, t, $J = 7.8$ Hz, H-8), 8.15 (1H, d, $J = 7.8$ Hz, H-7), 8.31 (1H, s, H-6), 8.56 (1H, d, $J = 7.7$ Hz, H-9), 8.66 (1H, s, H-4); ^{13}C NMR (CDCl_3) δ 38.4 (CH_2 , C-1'), 45.8 (CH_3 , C-4', C-5'), 55.2 (CH_3 , OCH_3), 57.1 (CH_2 , C-2'), 87.0 (C, C-2''), 92.2 (C, C-1''), 114.5 (CH, C-3''), 114.7 (C, C-1''), 122.8 (C, C-5), 123.4 (C, C-3a, C-9a), 127.3 (C, C-9b), 127.8 (CH, C-8), 131.5 (CH, C-7), 131.7 (C, C-6a), 133.5 (CH, C-2''), 133.7 (CH, C-9), 133.9 (CH, C-6), 135.4 (CH, C-4), 160.1 (C, C-OMe), 163.7 (C, C-3), 164.0 (C, C-1); EIMS m/z (%) 398 (31), 397 (1), 354(2) 327 (7), 71(38), 58 (100); HREIMS 398.1645 (calcd for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_3$ $[\text{M}]^+$ 398.1630); IR ν_{max} 2929, 2853, 2816, 2764, 2390, 2305, 2212, 2113, 1993, 1693, 1655, 1598, 1573, 1511, 1459, 1426, 1377, 1330, 1304, 1246, 1168, 1032, 929, 906, 828 cm^{-1} .

4.1.8. 2-(2-(Dimethylamino)ethyl)-5-((4-hydroxyphenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**10**)

Compound **10** was obtained from 62.0 mg of 3-iodonaphthalimide (0.16 mmol) following the general procedure described above after stirring for 8 h at room temperature. The crude product was purified by alumina preparative-TLC using EtOAc/hexanes (2:3)

to yield 12.9 mg (21%) of compound **10** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.54 (6H, s, H-5', H-4'), 3.03 (2H, t, J = 4.8 Hz, H-2'), 4.47 (2H, t, J = 5.1 Hz, H-1'), 5.88 (2H, d, J = 8.4 Hz, H-3''', H-5'''), 6.98 (2H, d, J = 8.5 Hz, H-2''' + H-6'''), 7.77 (1H, t, J = 8.0 Hz, H-8), 8.09 (1H, d, J = 1.3 Hz, H-6), 8.14 (1H, dd, J = 0.9, 8.6 Hz, H-7), 8.18 (1H, d, J = 1.4 Hz, H-4), 8.59 (1H, dd, J = 1.0, 7.2 Hz, H-9); ^{13}C NMR (CDCl_3) δ 37.4 (CH_2 , C-1'), 45.3 (CH_3 , C-4', C-5'), 57.1 (CH_2 , C-2'), 86.5 (C, C-2''), 92.4 (C, C-1''), 112.7 (CH, C-1'''), 114.8 (CH, C-2'''), C-5'''), 122.5 (C, C-5), 122.6 (C, C-9b), 123.2 (C, C-3a), 126.9 (C, C-9a), 127.2 (CH, C-8), 131.0 (CH, C-7), 131.3 (C, C-6a), 132.9 (CH, C-2'', C-6''), 133.1 (CH, C-9), 133.5 (CH, C-6), 135.0 (CH, C-4), 157.5 (C, C-4'), 164.2 (C, C-1 or C-3), 164.6 (C, C-1 or C-3); EIMS m/z (%) 384 (12), 326 (2), 296 (1), 242 (1), 134 (1), 71 (33), 58 (100); HREIMS 384.1474 (calcd for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_3$ [M] $^+$ 384.1474); IR ν_{max} 3595, 2958, 2920, 2852, 2203, 1654, 1601, 1329, 1236, 1159, 1015, 830, 780, 759, 745 cm^{-1} .

4.1.9. 2-(2-(Dimethylamino)ethyl)-5-((3-nitrophenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**11**)

Compound **11** was obtained from 62 mg of 3-iodonaphthalimide (0.16 mmol) following the general procedure described above after stirring for 12 h at room temperature. The crude product was purified by alumina preparative-TLC using EtOAc/hexanes (2:3) to yield 10 mg (15%) of compound **11** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.37 (6H, s, H-5', H-4'), 2.67 (2H, t, J = 6.9 Hz, H-2'), 4.34 (2H, t, J = 6.9 Hz, H-1'), 7.60 (1H, t, J = 8.0 Hz, H-5'''), 7.79 (1H, t, J = 7.7 Hz, H-8), 7.89 (2H, d, J = 7.6 Hz, H-7), 8.20 (1H, d, J = 8.2 Hz, H-4'''), 8.24 (1H, d, J = 8.2 Hz, H-6'''), 8.39 (1H, s, H-4), 8.43 (1H, s, H-2''), 8.60 (1H, d, J = 7.2 Hz, H-9); ^{13}C NMR (CDCl_3) δ 38.4 (CH_2 , C-1'), 45.8 (CH_3 , C-5', C-4'), 57.8 (CH_2 , C-2'), 89.2 (C, C-2''), 90.4 (C, C-1''), 121.4 (C, C-5), 122.9 (C, C-1'''), 123.4 (C, C-3a), 123.6 (CH, C-4'''), 124.5 (C, C-9a), 126.7 (CH, C-2'''), 127.8 (C, C-6b), 127.9 (CH, C-5'''), 129.7 (CH, C-6'''), 131.4 (C, C-6a), 132.1 (CH, C-8), 133.6 (CH, C-7), 133.8 (CH, C-9), 136.7 (CH, C-6), 137.5 (CH, C-4), 145.5 (C, C-NO₂), 163.7 (C, C-3), 164.0 (C, C-1); EIMS m/z (%) 414 (1), 413 (13), 384 (43), 354 (3), 327 (15), 71(43), 58 (100); HREIMS 413.1369 (calcd for $\text{C}_{24}\text{H}_{19}\text{N}_3\text{O}_4$ [M] $^+$ 413.1376); IR ν_{max} 3083, 2928, 2855, 2819, 2782, 2249, 2119, 1698, 1656, 1596, 1527, 1460, 1426, 1347, 1304, 1238, 1172, 1130, 1076, 1051, 1022, 981, 896, 854 cm^{-1} .

4.1.10. 2-(2-(Dimethylamino)ethyl)-5-((3-trifluoromethylphenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**12**)

Compound **12** was obtained from 31.5 mg of 3-iodonaphthalimide (0.08 mmol) following the general procedure described above after stirring for 3 h at room temperature. The crude product was purified by alumina preparative-TLC using EtOAc/hexanes (2:3) to yield 21.5 mg (63%) of compound **12** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.38 (6H, s, H-5', H-4'), 2.69 (2H, t, J = 5.5 Hz, H-2'), 4.36 (2H, t, J = 5.4 Hz, H-1'), 7.55 (1H, t, J = 7.7 Hz, H-5'''), 7.66 (1H, d, J = 7.7 Hz, H-4'''), 7.78 (1H, d, J = 7.7 Hz, H-6'''), 7.80 (1H, t, J = 7.8 Hz, H-8), 7.88 (1H, bs, H-2''), 8.20 (1H, d, J = 7.8 Hz, H-7), 8.38 (1H, d, J = 1.4 Hz, H-4), 8.61 (1H, dd, J = 7.4, 0.9 Hz, H-9), 8.69 (1H, d, J = 1.4 Hz, H-4); ^{13}C NMR (CDCl_3) δ 38.4 (CH_2 , C-1'), 45.8 (CH_3 , C-5', C-4'), 57.1 (CH_2 , C-2'), 89.4 (C, C-2''), 90.1 (C, C-1''), 121.9 (C, C-5), 122.9 (C, C-9b), 123.6 (C, C-3a), 123.6 (C, C-1'''), 125.6 (C, C-3'''), J = 28.0 Hz), 127.7 (CH, C-9b), 127.8 (CH, C-2'''), 129.2 (CH, C-8, C-5'''), 131.6 (C, C-6a), 132.0 (CH, C-4), 133.7 (CH, C-6'''), 133.8 (CH, C-6), 134.9 (CH, C-9), 136.5 (C, C-7), 163.7 (C, C-1 or C-3), 164.0 (C, C-1 or C-3); EIMS m/z (%) 436 (37), 378 (53), 277 (63), 178 (99) 71 (26), 58 (100); HREIMS 436.1400 (calcd for $\text{C}_{25}\text{H}_{19}\text{N}_2\text{O}_2\text{F}_3$ [M] $^+$ 436.1399); IR ν_{max} 3747, 3082, 2952, 2856, 2822, 2777, 2117, 1915, 1699, 1658, 1623, 1593, 1430, 1380, 1333, 1291, 1271, 1239, 1216, 1159, 1123, 1068, 1022, 979, 929, 897, 868 cm^{-1} .

4.1.11. 2-(2-(Dimethylamino)ethyl)-5-((furanethylphenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**13**)

A solution of 31.5 mg of 3-iodonaphthalimide (0.08 mmol) in toluene was treated with 14 μL of 3-ethynylfuran (0.08 mmol), 145 μL (0.96 mmol) of DBU, 4.0 mg of CuCl (0.04 mmol), 9.2 mg of Pd(P(PH_3))₄ and 40 mol% of H₂O. The reaction mixture was stirred at room temperature in the absence of light for 26 h, then, the reaction mixture was partitioned in ethyl ether and distilled water. The organic layer was washed with 10% HCl, saturated aqueous NaCl, dried over MgSO₄, filtered and the solvent removed under vacuum. The crude product was purified by alumina preparative-TLC to yield 5.2 mg (18%) of compound **13** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.36 (6H, s, H-5', H-4'), 2.67 (2H, t, J = 7.0 Hz, H-2'), 4.35 (2H, t, J = 6.9 Hz, H-1'), 6.59 (1H, d, J = 3.0 Hz, H-5'''), 7.47 (1H, m, H-4'''), 7.78 (2H, m, H-2'', H-8), 8.16 (1H, d, J = 7.5 Hz, H-7), 8.31 (1H, d, J = 1.3 Hz, H-6), 8.59 (1H, dd, J = 1.0, 7.4 Hz, H-9), 8.65 (1H, d, J = 1.5 Hz, H-4); ^{13}C NMR (CDCl_3) δ 38.1 (CH_2 , C-1'), 45.6 (CH_3 , C-5', C-4'), 56.9 (CH_2 , C-2'), 83.1 (C, C-2''), 89.6 (C, C-1''), 107.1 (C, C-1'''), 112.4 (CH, C-5'''), 122.4 (C, C-9b), 122.7 (C, C-3a), 122.9 (C, C-9a), 127.3 (C, C-6a), 127.5 (CH, C-8), 131.5 (CH, C-9), 133.4 (CH, C-7), 133.6 (CH, C-6), 135.8 (CH, C-4), 143.1 (CH, C-4'''), 146.2 (CH, C-2'''), 163.6 (C, C-3), 163.9 (C, C-1); EIMS m/z (%) 358 (16), 318 (12), 303 (8), 71 (56), 58 (100); HREIMS 358.1230 (calcd for $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_3$ [M] $^+$ 358.1317); IR ν_{max} 3750, 3115, 2928, 2856, 2773, 2344, 2221, 2120, 1997, 1921, 1702, 1659, 1628, 1600, 1434, 1336, 1292, 1243, 1157, 1030, 944, 908, 875, 825 cm^{-1} .

4.1.12. 2-(2-(Dimethylamino)ethyl)-5-((thiophenethylphenyl)ethynyl)-1H-benzo[de]isoquinoline-1,3-(2H)-dione (**14**)

A solution of 31.5 mg of 3-iodonaphthalimide (0.08 mmol) in toluene was treated with 28.8 mg of 3-ethynylthiophene (0.08 mmol), 145 μL (0.96 mmol) of DBU, 4.0 mg CuCl (0.04 mmol), 9.2 mg Pd(P(PH_3))₄ and 40 mol% of H₂O. The reaction mixture was stirred at room temperature in the absence of light for 24 h, then the reaction mixture was partitioned in ethyl ether and distilled water. The organic layer was washed with 10% HCl, saturated aqueous NaCl, dried over MgSO₄, filtered and the solvent removed under vacuum. The crude product was purified by alumina preparative-TLC to yield 8.4 mg (28%) of compound **14** as an amorphous yellow solid. ^1H NMR (CDCl_3) δ 2.38 (6H, s, H-5', H-4'), 2.68 (2H, t, J = 7.2 Hz, H-2'), 4.35 (2H, t, J = 7.2 Hz, H-1'), 6.46 (1H, s, H-5'''), 7.35 (1H, d, J = 1.2 Hz, H-2''), 7.64 (1H, s, H-4'''), 7.76 (1H, t, J = 6.5 Hz, H-8), 8.10 (1H, d, J = 6.6 Hz, H-7), 8.59 (2H, m, H-6, H-9), 8.81 (1H, s, H-4); ^{13}C NMR (CDCl_3) δ 38.2 (CH_2 , C-1'), 45.7 (CH_3 , C-5', C-4'), 56.9 (CH_2 , C-2'''), 87.0 (C, C-2'), 87.5 (C, C-1'), 121.6 (C, C-5), 122.5 (C, C-9a), 122.7 (C, C-3a), 122.9 (C, C-6a), 125.6 (C, C-1'''), 125.7 (CH, C-4'''), 127.3 (C, C-9b), 127.5 (CH, C-2'''), 129.6 (CH, C-8), 129.8 (CH, C-5'''), 131.5 (CH, C-9), 133.4 (CH, C-7), 133.7 (CH, C-6), 135.9 (CH, C-4), 163.6 (C, C-1 or C-3), 163.9 (C, C-1 or C-3); EIMS m/z (%) 374 (16), 349 (20), 304 (8), 71 (70), 58 (100); HREIMS 374.1138 (calcd for $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$ [M] $^+$ 374.1089); IR ν_{max} 3105, 2952, 2921, 2852, 2816, 2767, 2340, 2217, 2119, 1903, 1698, 1656, 1621, 1595, 1461, 1430, 1407, 1331, 1286, 1236, 1173, 1044, 1024, 986, 939, 869 cm^{-1} .

4.2. Molecular modeling studies

Docking studies were performed using the Glide 6.8. The PDB structure 1CX3 (d(ATGCAT)₂ duplex (D6)) as a template and the starting coordinates were taken from the Protein Data Bank (www.rcsb.org). DNA structures were prepared using preparation wizard for molecular docking as follows; hydrogen atoms were added, hydrogen bonding network was optimized, and protein was minimized to RMSD (Root Mean Square Deviation) 0.30 Å using

OPLS (Optimized Potential for Liquid Simulations) 2005 force field using Maestro 10.3 as graphical interface before docking study. A receptor grid was generated using a 1.00 van der Waals (vdW) radius scaling factor and 0.25 partial charge cutoff. The radius of 30 Å from the central atom of DNA was defined as the binding site that covers the entire DNA. Ligands were prepared using Lig-Prep 3.5 as implemented in Maestro 10.3 and were docked using the extra precision mode (XP) without using any constraints and a 0.80 van der Waals (vdW) radius scaling factor and 0.15 partial charge cutoff and using GlideScore for ligand ranking. A modified version of ChemScore,²⁵ GlideScore implemented in Glide, was used to estimate binding affinity and rank ligands. One poses per ligand were generated and post-docking minimization was carried out. Visualization of the docked poses and all 3D models has been carried out by using CHIMERA molecular graphics program.²⁶

4.3. Biological assays

The solvent for most stocks of the chemical agents employed was Dimethyl Sulphoxide (DMSO), especial Molecular Biology grade (DNase and RNase-free), from Sigma-Aldrich. Etoposide was purchased from Sigma-Aldrich and stored as a 10 mM stock in DMSO at -80 °C. Amonafide and all ethynylarylnaphthalimides were also stored in DMSO as a 10 mM stock at -80 °C until their use.

4.3.1. MTT cell viability assay

The human cancer cell lines HL60 (promyelocytic leukemia), HEL (human erythroleukemia) and SK-Br3 (breast adenocarcinoma) were purchased from ATCC and cultured in RPMI medium 10% FBS. The MTT assay, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], was used to test cytotoxicity of naphthalimides and cell viability.²⁷ Briefly, cells were plated in 96-well plates at 10,000 cells/well. Six hours after plating for HEL and HL60 cells and sixteen hours for SKBR3, vehicle (0.1% DMSO, final concentration) or compound was added to cells at indicated concentrations. Forty-eight hours following compound addition, MTT (Sigma-Aldrich, St. Louis, MO) was added to each well (0.3 mg/ml, final concentration) and plates were incubated for an additional 2 h at 37 °C. Medium was then aspirated and the formazan product was solubilized in SDS-HCl (20% SDS; HCl 0.02 M). The absorbance of each well was measured at 595 nm using an iMark Microplate Reader (BioRad, CA, USA). Non linear regression analysis was performed to calculate IC₅₀ according to the GraphPad Prism 5 program (GraphPad Software, San Diego, CA). The data are expressed by mean ± SD (n = 3).

4.4. Top2-mediated DNA relaxation and cleavage assays

The hTopoII α enzyme was purchased from Inspiralis (Norwich, UK). The reaction conditions were as described before.^{28,29,14} The substrate for the reaction was the plasmid pRYG purchased from TopoGEN (Columbus, OH). The amount of the hTopoII α enzyme was 2 units for the relaxation assay and 10 units for the cleavage assay. The incubation time was 30 min for the relaxation assay and 10 min for the cleavage assay. The incubation temperature was 37 °C. Inhibition of the hTopoII α relaxation activity was measured as normalized percentage of the substrate (SC form) that remains after the reaction. In all cases, the reaction reached completion in just the vehicle 1% (v/v) DMSO.

4.5. Dose-response yeast growth curves

Yeast strains FM588 and FM888 were used as the reference wild type (*RAD52*) and its *rad52* Δ counterpart, respectively, and have

been described before.^{14,28} For the assay, both strains were grown overnight at 25 °C to log phase (OD₆₂₀ of 0.4–0.8), then diluted to OD₆₂₀ of 0.01 (~10⁵ cells/mL) and aliquoted into 96-well flat bottom plates. Tested compounds were added in eight 1:2 serial dilutions ranging from 1 to 128 μ M in DMSO 1% (v/v). The vehicle alone was also used as a control. Growth was measured by OD₆₂₀ readings after 24 h incubation at 25 °C without shaking. Relative growth to just DMSO 1% (v/v) was then calculated and plotted against the logarithm of drug concentrations.

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2017.02.024>.

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